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Pollen segmentation and feature evaluation for automatic classification in bright-field microscopy

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ABSTRACT

Besides the well-established healthy properties of pollen, palynology and apiculture are of extreme importance to avoid hard and fast unbalances in our ecosystems. To support such disciplines computer vision comes to alleviate tedious recognition tasks. In this paper we present an applied study of the state of the art in pattern recognition techniques to describe, analyze, and classify pollen grains in an extensive dataset specifically collected (15 types, 120 samples/type). We also propose a novel contour–inner segmentation of grains, improving 50% of accuracy. In addition to published morphological, statistical, and textural descriptors, we introduce a new descriptor to measure the grain's contour profile and a logGabor implementation not tested before for this purpose. We found a significant improvement for certain combinations of descriptors, providing an overall accuracy above 99%. Finally, some palynological features that are still difficult to be integrated in computer systems are discussed.

1. Introduction

A grain of pollen contains the male vegetative and generative cells required for fertilization of plants to ensure the development of seeds and consequently the life of plants. The study of pollen, palynology, is therefore of great interest in so diverse disciplines such as archeology, paleontology, forensics, health (allergies) or agriculture (bee products, and crop forecast). Specifically, bee pollen is collected by worker honey bees which is used as food for the entire colony. For humans it is one of the richest and purest natural foods, with an incredible nutritional and medicinal value (Bogdanov et al.; Bogdanov) and one of the most interesting facts about bee pollen is that it cannot be synthesized in a laboratory. The main nectar source and main pollen source differ widely with latitude, region, season, and type of vegetation, where in scarce nectar periods bees can harvest far away up to 3 km, i.e., in an area of 300–2800 hectares (Eckert, 1933). This reflects their large pollination capacity and the maintenance of plant diversity which directly influences important human activities like agricultural and forestry production. Furthermore, bees are the most common pollinators with strong influence on ecological relationships, ecosystem conservation, and stability, genetic variation in the plant community, biodiversity, specialization, and evolution (Bradbear et al., 2009).

The pollen grains manifest a great variety of shapes, sizes, and ornamentation and their description is genetically bound to their botanical family. Externally, pollen grains are protected by a resistant wall called sporoderm, conformed by an internal layer named intine and an outer layer named exine, where the latter exhibits in its surface distinct morphological structures according to the pollen type. Generally, most of them are spheroidal in equatorial view, varying between oblate spheroidal and prolate spheroidal in the range of 8–100 μm.

In the human activities previously mentioned a correct pollen identification is vital in terms of production, bio-preservation, or simply knowledge achievement. The recognition can be accomplished through different techniques which in general are time consuming and require highly trained palynologists who must

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Table 1
Pollen database description.

Pollen types	*Aster, Brassica, Campanulaceae, Carduus, Castanea, Cistus, Cytisus, Echium, Ericaceae, Helianthus, Olea, Prunus, Quercus, Salix, Teucrium*
Magnification	$\times 40$
Original captures	2560×1920 RGB pixels
Cropped grains	From 200 to 600 gray pixels of width and height (variable aspect ratio)
Type grains	120 images/type
Total samples	1800 images (grains)

analyze manually thousands of individual pollen grains: Fourier transformed infra-red from attenuated total reflectance (FTIR-ATR) spectroscopy represents a useful technique for identifying chemical structures (Gomez-Ordonez and Ruperez, 2011); and Polymerase Chain Reaction (PCR) is a recent method for pollen authenticity based on molecular analysis. PCR technique stands out for its specificity for botanical identification. Nevertheless both techniques are expensive in terms of equipment and reagents, and requires several processing days. Finally, the most common and affordable technique is bright-field microscopy. This technique is time consuming too and therefore many efforts have been put on automated classification systems. However it remains a challenge to provide accurate pollen classifications in real scenarios. For a recent study that provides a comparison of the microscopy techniques, see Sivaguru et al. (2012).

The first attempt to automate pollen recognition was conducted in 1968 by Flenley (1968), who identified two difficulties attached to bright-field microscopy: images partially focused and multiple grain orientations (views). Both are related with the reduction of 3D objects into 2D captures. The depth of field of optical systems allows visualization of specimens partially in focus. Here, the use of multifocus stacks and recent multifocus fusion techniques (Redondo et al., 2009) could eventually provide more details about pollen's surface, but the way of collecting information is still an open issue. On the other hand, morphology, surface ornamentation, and pori layout are strong indicators of the pollen type, but such information strongly vary with the point of view.

Besides these inherent difficulties in capturing 3D features into 2D, two main obstacles hamper the current progress in this field: (a) the extraction of knowledge from expert palynologists and (b) the limited access to open pollen databases with a large number of reference pollen per taxa. A previous work in the area of aeropalynology (ASTHMA EU project) used multifocus stacks and reported recognition rates around 97% for 5 pollen types (Boucher et al., 2004). Other studies demonstrate accuracy ratios between 90% and 97% (Rodriguez-Damian et al., 2006; Chen et al., 2006; Ticay-Rivas et al., 2011; Chica, 2012; Ronneberger et al., 2007). However, such ratios must be considered with care, they are not reliably comparable because their training database usually differ largely in terms of pollen genre and/or number of training samples, which is directly related to obstacle (b).

Most of these approaches, if not all, perform morphological and certain statistical description of gray-levels like mean, median, variance, entropy, etc. Some modern approaches incorporate more sophisticated descriptors through spatial correlations like the Haralick's co-occurrence matrices (Haralick et al., 1973). For instance, Zhang proposed Gabor transforms and invariant moments (Zhang et al., 2004), Rodriguez-Damian et al. (2004) evaluated Fourier descriptors and Run-Length Statistics, Chen et al. (2006) incorporated a description of the number of pores and recently Ronneberger et al. proposed 3D invariant moments (Ronneberger et al., 2007). An interesting and profuse thesis can be consulted in Haas (2011).

In some applications, e.g. images from ambient air, a previous image cleaning from dirt, fungal spores and other non-pollen particles (Landsmeer et al., 2009) is required. This is also a time consuming process where a robust automatic segmentation is a challenging problem.

In this paper we present a complete applied study of segmentation, description, and classification of bee pollen, reviewing the state of art and proposing some novel techniques. For that, within the EU-funded project APIFRESH, we recollected an important data base of 15 pollen types with 120 samples per type described in Section 2. Under the hypothesis that contour and inner of grains typically manifest disparate statistical distributions, we proposed in Section 3 a novel segmentation to apply descriptors separately across these two regions. In Section 4 an important exercise of knowledge transfer is done from palynology to computer vision together with a complete list of descriptors. Sections 5 and 6 describe classification strategies and classifiers. Finally experimental results are presented in Sections 7 and 8 concludes the paper addressing unresolved challenging problems.

2. Materials and preparation: collecting database

Bees collect pollen aggregated in balls and normally of the same pollen type, which guarantees a certain corresponding hue. Therefore, balls were separated in the laboratory and individualized by color tonality and then labeled with a color code according to the Universal Code Guide PANTONE 747XR. Although pollen can share color, each color corresponds to a pollinic type and a pollinic type can be matched to a larger group of plants (a family), to a middle group of plants (some genera from the same family), to a reduced group of plants (a genus) or more rarely to one species. Balls collected from the same place of origin were classified in colors and for each color we selected 25 pollen balls. Balls were dissolved with glycerogelatin drops and prepared in slices sealed with a coverslip. Through the microscope each botanical group has characteristic features that differentiate it from others like morphology, surface structures or pori layout. For a summarized featured list of the pollen types studied here consult the appendix in Appendix A.

Although multiple studies have already evaluated a wide range of pollen descriptors, most of them have been done with a reduced dataset and/or a reduced number of pollen types. Without a doubt one of the major efforts in this kind of studies has to do with the compilation, preparation and labeling of datasets. Thus, some of those studies deal with 300–500 total samples and/or 3–5 pollen types (Rodriguez-Damian et al., 2006; Chen et al., 2006; Boucher et al., 2004; Carrión et al., 2002; Travieso et al., 2011; Ticay-Rivas et al., 2011). The study from Chica (2012) is one the most complete in this respect with 5 pollen types and 1063 total pollen grains. But one impressive case is Ronneberger et al. (2007) with 180 000 airborne particles and 22 700 pollen grains. In this case study, we have done an important effort to collect a considerable dataset in order to test computer vision algorithms focused on a real automated pollen classifier.

The 15 pollen types studied were collected mostly from Spain (Guadalajara, Toledo, La Rioja, Madrid and Cantabria). Other types like *Aster* and *Castanea* came from Italy (Grosseto, Cosenza and Asti), *Helianthus* from Bulgaria and *Teucrium* from Turkey. They are enumerated in Table 1 and some examples are depicted in